

Results from the Swedish
National Screening
Programme 2007

Subreport 3: Pigments

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Summary As an assignment from the Swedish EPA, IVL and NILU has during 2007/2008 developed analytical methods and performed a "Screening Study" of selected pigments. Since no previous measures of pigments in the Swedish environment have been made, the overall objective of the screening was to determine if the selected pigments are present, and if so, determine their concentrations in a variety of media in the Swedish environment. In total, 51 samples were analysed representing soil, surface water, sediment, biota (fish), sludge and influent and effluent water from municipal STPs, storm water, leachate water and leachate water sludge from landfills, effluent water from public laundries, and process water and sludge from a deinking plant treating recycled paper. The pigments included in the study were Pigment yellow 1, Pigment orange 5, Pigment red 53:1 and Pigment red 170. All four pigments were found in influent waters from the municipal STPs, but only Pigment red 53:1 could be found in the effluent water and this only in one out of four samples. Three of the pigments, Pigment red 53:1, red 170 and yellow 1, could also be found in sludge. Thus removal of the pigments takes place during the waste water treatment process. All four Pigments could also be found in storm water samples from the city of Stockholm. Diffuse emissions from the society thus do occur. Two of the pigments, Pigment red 53:1 and Pigment red 170, could be found in sediment samples from the city of Stockholm and from the recipient of one of the STPs. All four pigments could be found in effluent water from a public laundry and in samples from different process stages of the deinking of recycled paper in a paper mill. This indicates that textiles and paper products may be sources to the diffuse emissions seen. None of the pigments could be detected in the samples from background areas. Emissions in and transport to rural areas thus seems to be minor.	
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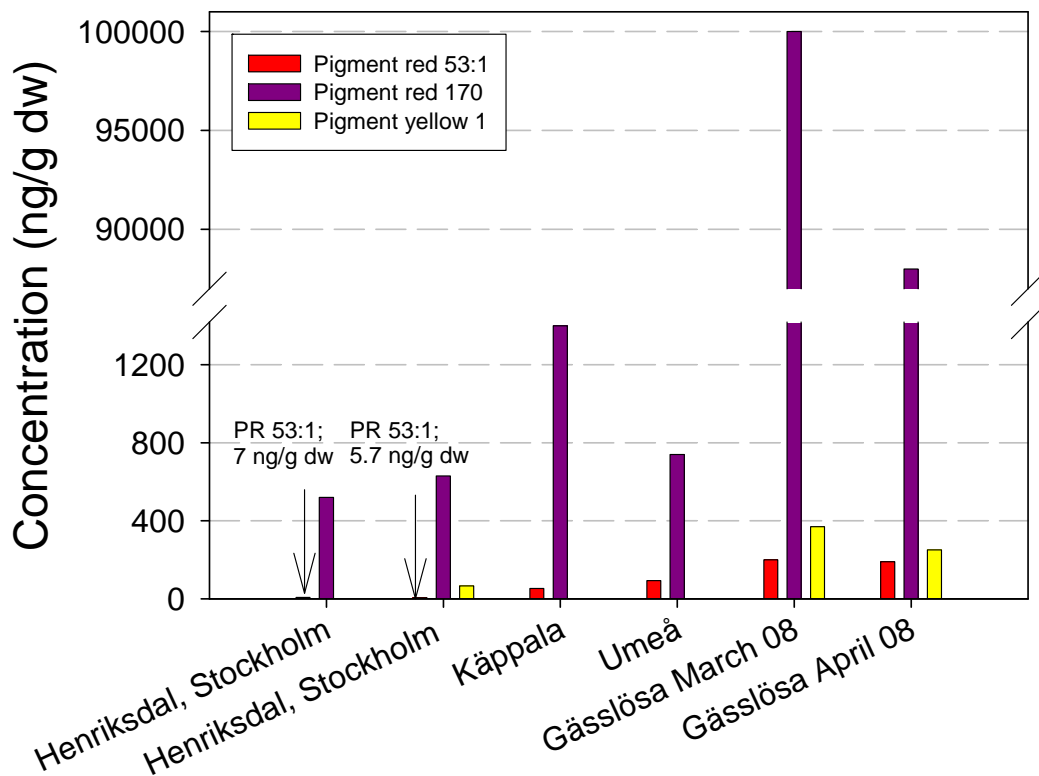
Sammanfattning

IVL Svenska Miljöinstitutet AB och Norsk Institutt for Luftforskning (NILU) har på uppdrag av Naturvårdsverket utvecklat analysmetoder för, samt genomfört en screening av, pigmenter. Ursprungligen ingick det sju olika pigmenter i studien. För fyra av pigmenterna, Pigment gul 1, Pigment orange 5, Pigment röd 53:1 samt Pigment röd 170, kunde analysmetoder tas fram. För de resterande tre, Pigment violett 23, Pigment blå 15 samt Pigment grön 7, lyckades ej utvecklingen av analysmetoder och de har därför exkluderats ur screeningen.

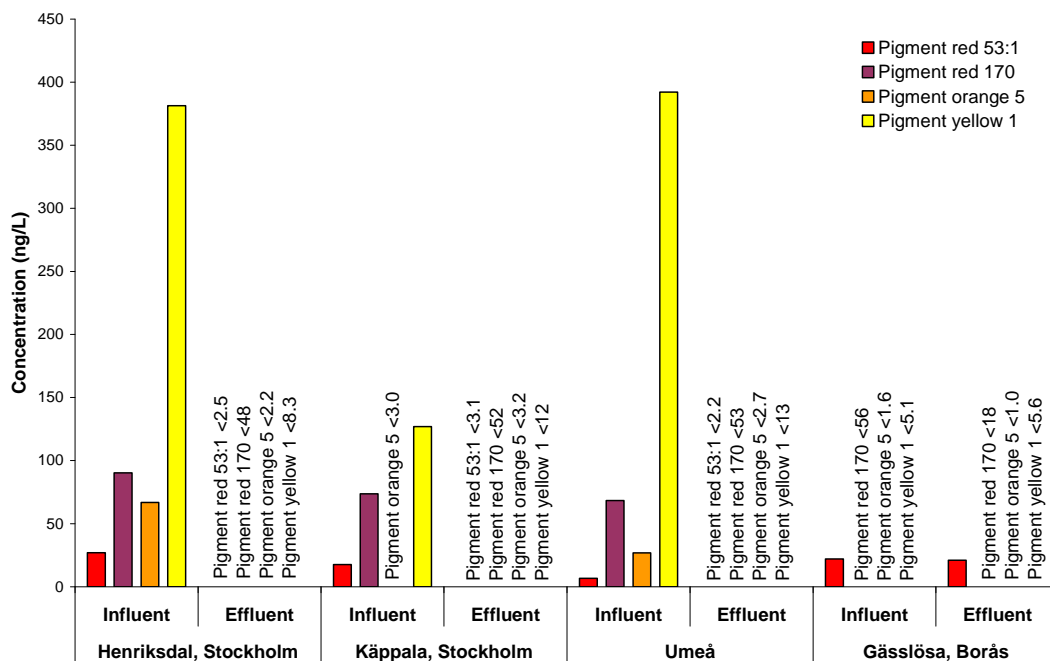
De analyserade pigmenterna är alla syntetiska organiska azoföreningar vilka karakteriseras av en kromator azo-grupp (-N=N-). De är högmolekylära, har låg vattenlöslighet, och förväntas ha relativt hög persistens i miljön. Azoföreningars toxicitet relateras huvudsakligen till carcinogena aromatiska aminer som kan bildas vid nedbrytning. 22 av dessa aminer är listade i det 19e tillägget till EU-direktiv 76/769/EEC, vilket reglerar användning av färgämnen som kan ge upphov till dessa. Inget av de pigment som ingår i denna screening kan dock ge upphov till någon av de 22 aminerna.

Då inga tidigare studier avseende förekomst i miljön av de ingående pigmenterna har gjorts, var syftet med den föreliggande studien att utreda huruvida pigmenterna förekommer i den svenska miljön, och om så, i vilken omfattning. En provtagningsstrategi utarbetades utifrån en kartläggning av potentiella källor, samt pigmenternas predikterade egenskaper och fördelning i miljön. Utifrån denna valdes provtagningsplatser som representerade punktkällor, diffusa källor samt bakgrundsområden. Totalt bestämdes pigmenten i 51 prover. Dessa bestod av ytvatten, sediment, fisk, jord, ingående och utgående avloppsvatten, avloppsslam, dagvatten, lakvatten och lakvattenslam från deponier, avloppsvatten från tvätterier, samt processvatten och processvattenslam från en returpappersanläggning.

Uppmätta halter i slam samt i ingående och utgående avloppsvatten presenteras nedan i Figur 1 och Figur 2. Tre av de analyserade pigmenten uppmättes i koncentrationer över detektionsgränsen i slam. Pigment röd 170 uppmättes i de högsta koncentrationerna och detta pigment påträffades i samtliga analyserade slamprover. Även Pigment röd 53:1 och Pigment gul 1 påträffades i slamproverna, Pigment röd 53:1 i slam från samtliga och Pigment gul 1 i slam från två av fyra ingående reningsverk. De högsta halterna pigment uppmättes i slam från Gässlösa reningsverk. För Pigment röd 170 var halterna ungefär 1000 gånger högre jämfört med de övriga reningsverken. Screeningprogrammet kompletterades därför med ingående och utgående avloppsvatten från Gässlösa, samt ytvatten och sediment nedströms och sediment uppströms i avloppsverkets recipient Viskan. Förekomst av alla de analyserade pigmenten uppmättes i ingående avloppsvatten, medan endast Pigment röd 53:1 uppmättes i utgående avloppsvatten och detta bara i provet från Gässlösa, se Figur 2. Då slam från Gässlösa innehöll betydligt högre halter av pigmenten än slam från de övriga reningsverken, förväntades höga halter även detekteras i de kompletterande avloppsvattenproverna. Detta var dock inte fallet, endast Pigment röd 53:1 uppmättes i det ingående vattnet från Gässlösa. Vattenproverna från Gässlösa togs dock ej vid samma tillfälle som de övriga proverna utan under augusti 2008. Skillnaderna i pigmentförekomst för avloppsvatten mellan Gässlösa och de övriga avloppsverken, samt mellan vad som uppmättes för Gässlösa respektive var förväntat utifrån halterna i slam skulle därför kunna bero på säsongsvariationer. Gässlösa reningsverk tar emot avloppsvatten från industrier som använder pigmenter. Det är möjligt att tillförseln till reningsverket sker i pulser, alternativt varierar över året.



Figur 1. Koncentrationer av pigmenter i slam från avloppsverk.



Figur 2. Koncentrationer av pigmenter i ingående och utgående avloppsvatten.

Två av pigmenten, Pigment röd 53:1 och Pigment röd 170, uppmättes i sediment från recipienten till Gässlösa avloppsverk, se Tabell 1. Halterna var lite högre i sedimentprovet taget uppströms än i provet taget nedströms. Utsläpp via reningsverket kan därför inte vara enda källan till förekomsten i detta vattendrag.

Tabell 1. Pigmentkoncentrationer i ytvatten och sediment från Viskan, recipient till Gässlösa avloppsverk.

Prov	Enhet	Pigment röd 53:1	Pigment röd 170	Pigment orange 5	Pigment yellow 1
Ytvatten nedströms	ng/l	<1.8	<27	<1.1	<2.5
Sediment nedströms	ng/g tv	1.4	65	<2.0	<0.7
Sediment uppströms	ng/g tv	3.9	96	<2.0	<0.7

I tabell 2 visas halter av pigmenterna uppmätta i olika matriser från den urbana miljön. Samtliga analyserade pigmenter kunde bestämmas i dagvatten från centrala Stockholm. Två av pigmenterna, Pigment röd 53:1 och Pigment röd 170, återfanns i sediment och Pigment röd 53:1 även i ett ytvattenprov från centrala Stockholm.

Tabell 2. Koncentrationer av de analyserade pigmenterna i ytvatten, sediment, fisk samt dagvatten från Stockholm.

Provtagningsplats	Provtyp	Enhet	Pigment yellow 1	Pigment orange 5	Pigment röd 53:1	Pigment röd 170
Stora Essingen	Ytvatten	ng/l	< 12	< 2.9	< 3.9	< 85
	Sediment	ng/g tv	< 52	< 8.0	7.6	< 107
	Fisk	ng/g vv	< 16	< 3.3	< 1.5	< 88
Årstaviken	Ytvatten	ng/l	< 10	< 2.4	< 4.1	< 71
	Sediment	ng/g tv	< 29	< 8.5	5.9	190
	Fisk	ng/g vv	< 20	< 4.9	< 1.1	< 10
Riddarfjärden	Ytvatten	ng/l	< 20	< 2.1	5	< 69
	Sediment	ng/g tv	< 21	< 3.4	13	330
	Fisk	ng/g vv	< 18	< 2.8	< 1.0	< 80
Eugeniatunneln	Dagvatten	ng/l	< 17	< 1.8	< 4.0	< 77
Torstenssonsgatan	Dagvatten	ng/l	750	65	210	390
Sveavägen	Dagvatten	ng/l	< 68	59	370	2800

I Tabell 3 nedan visas resultaten för de identifierade punktkällorna. Samtliga pigmenter uppmättes i avloppsvatten från ett av tvätterierna. I provet från det andra tvätteriet kunde endast Pigment röd 53:1 detekteras. Detta prov togs dock efter att vattnet även passerat ett kommunalt reningsverk. Samtliga pigmenter kunde även detekteras i höga halter i olika steg i avfärgningsprocessen av returpapper i en massafabrik. I jordprovet taget på en båtklubb kunde två av pigmenten uppmätas, Pigment orange 5 och Pigment röd 170. Detta prov togs efter att vårunderhåll, vilket kan omfatta slipning och måleriarbeten, ägt rum. Inget av pigmenterna kunde identifieras i något av proverna tagna i anslutning till deponier.

Tabell 3. Pigmentinnehåll i prover från identifierade punktkällor.

Prov	Enhet	Pigment yellow 1	Pigment orange 5	Pigment red 53:1	Pigment red 170
Avloppsvatten tvätteri					
Södra Götaland	ng/l	190	98	85	1000
Svealand	ng/l	< 12	< 2.4	13	< 32
Massafabrik					
Process vatten steg 1	ng/l	61000	9800	450000	8900
Process vatten steg 2	ng/l	27000	3200	160000	6000
Slam 1	ng/g tv	800	650	4200	1600
Slam 2	ng/g tv	370	< 6.5	1100	420
Inj till flotationssteg	ng/g tv	< 142	< 41	1500	< 361
Årstavikens segelsällskap					
Jord	ng/g tv	< 0.6	6.8	< 0.3	130

Sammanfattningsvis visar mätningarna att det sker en diffus spridning av de studerade pigmenten i den svenska miljön. Detta indikeras av deras förekomst i avloppsvatten och avloppsslam, dagvatten, sediment, ytvatten samt jord.

Förekomst av pigmenten i prover från de olika stegen i avfärgningen av returpapper samt i avloppsvatten från ett tvätteri indikerar att papper och textilier är källor till spridning av pigmenten.

Inget av de analyserade pigmenten kunde detekteras i proverna från bakgrundsområden. Detta tyder på att spridning utanför den urbana miljön inte sker i någon större utsträckning.

Summary

As an assignment from the Swedish Environmental Protection Agency, IVL Swedish Environmental Research Institute and the Norwegian Institute for Air Research (NILU) has during 2007/2008 developed analytical methods and performed a "Screening Study" of selected pigments. Since no previous measures of pigments in the Swedish environment have been made, the overall objective of the screening was to determine if the selected pigments are present, and if so, determine their concentrations in a variety of media in the Swedish environment. The screening programme included measurements in background areas and in the vicinity of potential point sources. Measurements were also carried out in urban areas reflecting diffuse emission pathways from the society. Sample types were, soil, surface water, sediment, biota (fish), sludge and influent and effluent water from municipal STPs, storm water, leachate water and leachate water sludge from landfills, effluent water from public laundries, and process water and sludge from a deinking plant treating recycled paper. In total, 51 samples were included.

The pigments included in the study were Pigment yellow 1, Pigment orange 5, Pigment red 53:1 and Pigment red 170. For three pigments, Pigment violet 23, Pigment blue 15 and Pigment green 7, analytical methods could not be established and they were thus excluded from the screening.

The pigments are synthetic compounds characterized by chromophoric azo groups (-N=N-). They are high molecular compounds of low solubility expected to be relatively persistent in the environment. Several azo compounds may form carcinogenic aromatic amines during degradation, but the pigments included in this screening are not among those.

All four pigments were found in influent waters from the municipal STPs, but only Pigment red 53:1 could be found in the effluent water and this only in one out of four samples. Three of the pigments, Pigment red 53:1, red 170 and yellow 1, could also be found in sludge. Thus removal of the pigments takes place during the waste water treatment process. All four Pigments could also be found in storm water samples from the city of Stockholm. Diffuse emissions from the society thus do occur.

Two of the pigments, Pigment red 53:1 and Pigment red 170, could be found in sediment samples from the city of Stockholm and from the recipient of one of the STPs.

All four pigments could be found in effluent water from a public laundry and in samples from different process stages of the deinking of recycled paper in a paper mill. This indicates that textiles and paper products may be sources to the diffuse emissions seen.

None of the pigments could be detected in the samples from background areas. Emissions in and transport to rural areas thus seems to be minor.

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1 Introduction

As an assignment from the Swedish Environmental Protection Agency, IVL and the Norwegian Institute for Air Research (NILU) has during 2007/2008 performed a "Screening Study" of amines, esters, pigments, linear alkyl benzene sulfonate (LAS) and silver. These substances are emitted and distributed in the environment via a variety of sources, e.g. different point sources and/or diffusive sources. Some of them are used in consumer products.

The overall objectives of the screening studies are to determine the concentrations of the selected substances in a variety of media in the Swedish environment, to highlight important transport pathways, and to assess the possibility of current emissions in Sweden.

The results are given in five subreports according to Table 1.

Table 1. Substances / substance groups included in the screening

Substance / Substance group	Sub-report #
Amines:	1
3,6,9,12-Tetraazatetradecane-1,14-diamine	
N-cyclohexyl-2-benzothiazolamine (NCBA)	
N-isopropyl-N'-phenyl-p-phenylenediamine (IPPD)	
N-Phenyl-benzeneamine	
Dicyclohexylamine	
Esters:	2
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	
Pigments:	3
Pigment yellow 1 (CI 11680)	
Pigment orange 5 (CI 12075)	
Pigment red 53:1 (CI 15585:1)	
Pigment red 170 (CI 12475)	
Linear alkyl benzene sulfonate (LAS)	4
Silver	5

This subreport considers the screening of pigments.

The pigments included in the screening are all synthetic organic compounds characterized by chromophoric azo groups (-N=N-). Azo compounds of low solubility are called pigments, whereas compounds that dissolve during their application are called dyes. Azo-compounds are produced by a diazotization reaction (Møller and Wallin, 2000). A diazonium chloride moiety is formed by the reaction of a primary amine and sodium nitrite in hydrochloric acid, which then can be coupled to electron rich compounds by an electrophilic attack. By using different primary amines and electron rich compounds with varying functional groups, a wide range of pigments with different properties can be produced. Table 2 shows the molecular structure and some of the synonyms for the pigments included in this screening.

This screening started with the development of analytical methods for determination of the selected pigments in environmental samples, see chapter 6 and Appendix 2. Four of the seven pigments selected for the screening were possible to analyse, and they are shown in Table 2. For Pigment

violet 23 (cas no 6358-30-1), Pigment blue 15 (cas no 147-14-8) and Pigment green 7 (cas no 1328-53-6), analytical methods could not be established and they were thus excluded from the screening.

Table 2. Names, synonyms, CAS numbers and molecular structures of pigments included in the study.

Name	Synonyms	Cas no	Molecular structure
Butanamide, 2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenyl-	Pigment yellow 1, CI 11680, Hansa Yellow G	2512-29-0	
2-Naphtalenol, 1-[(2,4-dinitrophenyl)azo]-	Pigment orange 5, CI 12075	3468-63-1	
Benzenesulfonic acid, 5-chloro-2-[(2-hydroxy-1-naphthalenyl)azo]-4-methyl-, barium salt (2:1)	Pigment red 53:1, CI 15585:1	5160-02-1	
2-Naphtalenecarboxamide, 4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxy-	Pigment red 170, CI 12475	2786-76-7	

2 Chemical properties, fate and toxicity

2.1 Properties and fate

Documentation on the physico-chemical properties of the pigments included in the screening is scarce. In order to predict possible environmental fate and impact, physico-chemical data and estimates on half-lives in different matrices have been retrieved by structural modelling using the EPI Suite v3.20. Predicted data, which due to the uncertainty using models should be regarded as indicative, are presented in Table 3.

All of the included pigments have relatively high molecular weights and are solids at room temperature with melting temperatures above 200 °C. Volatisation of the compounds are thus unlikely to occur, which is also supported by the low modelled vapour pressures.

Table 3. Physico-chemical properties of the pigments included in the study. Data predicted by structural modelling using EPI Suite v3.20.

Property	Pigment yellow 1	Pigment orange 5	Pigment red 53:1 ^a	Pigment red 170
MW (g/mol)	340.34	338.28	888.94	454.49
Melting point (°C)	225	221	246 330 (decomp.) ^b	327
Boiling point (°C)	526	518	571	745
Vapour pressure	4.26E-11	9.32E-12	4.87E-15	1.45E-19
Water solubility (mg/L)	1.13	0.134	5.66 2 (exp. value) ^b	0.0135
Henry's law constant (Pa m ³ /mol)	1.68E-11	3.11E-11	4.26E-16	6.41E-18
Log Kow	3.94	5.72	3.54	6.04
Koc	279	49700	7140	34300
Half-lives t_{1/2} (days)				
Air	0.75	0.63	0.58	0.33
Water	60	60	60	60
Soil	120	120	120	120
Sediment	540	540	540	540

^a For Pigment red 53:1, all data except the molecular weight are retrieved by modelling the corresponding acid, not the barium salt.

^bOECD SIDS (2002)

All the pigments included in the screening are expected to be relatively stable in the environment. Predicted half-lives in soil are 120 days and in sediments 540 days, see Table 3. To our knowledge, pigment red 53:1 is the only of the pigments included in the screening for which there are experimental degradation data available. According to OECD SIDS (2002), Pigment red 53:1 is not biodegradable.

In general, azo compounds are relatively persistent under aerobic conditions, but can be degraded to aromatic amines under anaerobic conditions by reduction of the azo group (-N=N-) (van der Zee *et al.*, 2001). The formed aromatic amines can then be further degraded under aerobic conditions, but for some amines this requires enrichment of/with specialized bacteria (van der Zee *et al.*, 2005). Competing with the degradation of the formed aromatic amines, they may also be

autoxidised under aerobic conditions, forming different compounds that may be toxic such as azoxy compounds (van der Zee *et al.*, 2001).

Due to the relatively high log Kow- and Koc values, all of the pigments are predominantly expected to adsorb to solids such as soil and sediments, but non-insignificant proportions are also expected to be found in water. The predicted environmental distributions after equal releases to air, water and soil are presented in Table 4.

Table 4. Predicted distribution (%) after equal releases to air, water and soil. Fugacity model level III, EPI Suite v3.20.

Compartment	Pigment yellow 1	Pigment orange 5	Pigment red 53:1 ^a	Pigment red 170
Air	3E-7	3E-5	3E-4	0.03
Water	9	3	9	3
Soil	90	60	90	50
Sediment	2	40	0.9	50

^aDistribution modelled for the corresponding acid, not the barium salt.

2.2 Toxicity

The major concern regarding azo compounds is the toxicity of the aromatic amines formed during degradation. The reduction of azo compounds to aromatic amines do not only take place under anaerobic conditions in sewage treatments plants, but can also be promoted by azoreductases from intestinal bacteria, in liver cells and skin micro flora (Ahlström *et al.*, 2005). Several of these degradation products are known carcinogens, and for azo dyes generating any of 22 aromatic amines covered by the 19th amendment (directive 2002/61/EC) of EU-directive 76/769/EEC, their use in textiles and leather products that may come in direct contact with human skin or oral cavity is prohibited. Contrary to the azo dyes, the use of azo pigments is not restricted, and neither of the pigments included in the screening may generate any of the 22 listed aromatic amines.

Pigment yellow 1 is not mutagenic in Ames test but known to cause contact dermatitis in heavily exposed painters (Øllgard *et al.*, 1998).

The toxicity of pigment orange 5 has been evaluated by BG Chemie (2000). Animal studies show low acute toxicity, low chronic toxicity, and no teratogenic or sensitizing properties. It has been shown to be mutagenic in different Salmonella/microsome tests and to cause DNA damage in a comet assay with rat hepatocytes, but not in the DNA repair test nor in vivo. Chronic exposure of female rats had a weak hepatocarcinogenic effect/potential at concentrations also causing hepatotoxicity, and chronic exposure of male mice caused an increase of hepatocellular tumours but only at concentrations also causing general toxicity. According to Møller and Wallin (2000), Pigment orange 5 should be considered genotoxic.

Pigment red 53:1 has been evaluated by OECD SIDS (2002). It has low acute and chronic toxicity, and no teratogenic or sensitizing properties. The contact sensitizing properties earlier reported have been shown to be due to impurities, which has resulted in the development of a new manufacturing process. It is not genotoxic in vitro nor in vivo, but carcinogenic in male rats at concentrations also causing general toxicity.

For pigment red 170, no toxicological data at all were found in the literature.

Ecotoxicological data for the included pigments is even scarcer. The only literature data available indicate acute LC50-values for fish and Daphnia for Pigment red 53:1 above the water solubility OECD SIDS (2002). Using PBT-profiler (<http://www.pbtprofiler.net/>), the ECOSAR toxicity values presented in Table 5 were predicted.

Table 5. Most sensitive ECOSAR estimates for the pigments included in the screening (PBT-profiler).

Pigment	ECOSAR Class	Organism	Duration	Endpoint	Predicted value (mg/l)
Pigment yellow 1	Neutral organics	Daphnid	16 days	EC50	0.56
		Mysid shrimp	96 hrs	LC50	0.27
Pigment orange 5	Phenols	Fish	-	ChV	0.0017
		Fish	90 days	ChV	0.009
Pigment red 53:1^a	Phenols - acid	Fish	90 days	ChV	0.92
Pigment red 170	Phenols	Fish	90 days	ChV	0.008

^aDistribution modelled for the corresponding acid, not the barium salt.

ChV: Chronic Value

Human exposure to azo dyes can occur via different routes such as dermal absorption (e.g. after elution by sweat from clothes) and ingestion (e.g. children sucking on toys) (Ahlström *et al.*, 2005).

Other possible exposure pathways are through leachage from products into food stuffs, exposure to paper and printer dust, during use of paints and lacquers, and occupational during handling of pigments.

Absorption of pigments is expected to be low due to their low solubilities. Absorption of Pigment orange 5 has been shown to be less than 0.007% (BG Chemie, 2000), and Golka *et al.* (2004) concluded in a review on the bioavailability of pigments, that pigments based on 3,3'-dichlorobenzidine do not pose any carcinogenic risk, due to the low bioavailability associated with their low solubilities.

3 Consumption and emissions

The Swedish use of the pigments included in the screening between the years 1999 and 2005 is given in Figure 1 (Spin, 2008). Pigment red 53:1 was used in the largest amounts compared to the other pigment, while Pigment yellow 1 was used in the smallest amounts. The use of Pigment red 53:1 was 60-70 tonnes/year 2001-2005, and there was no decreasing trend during these years.

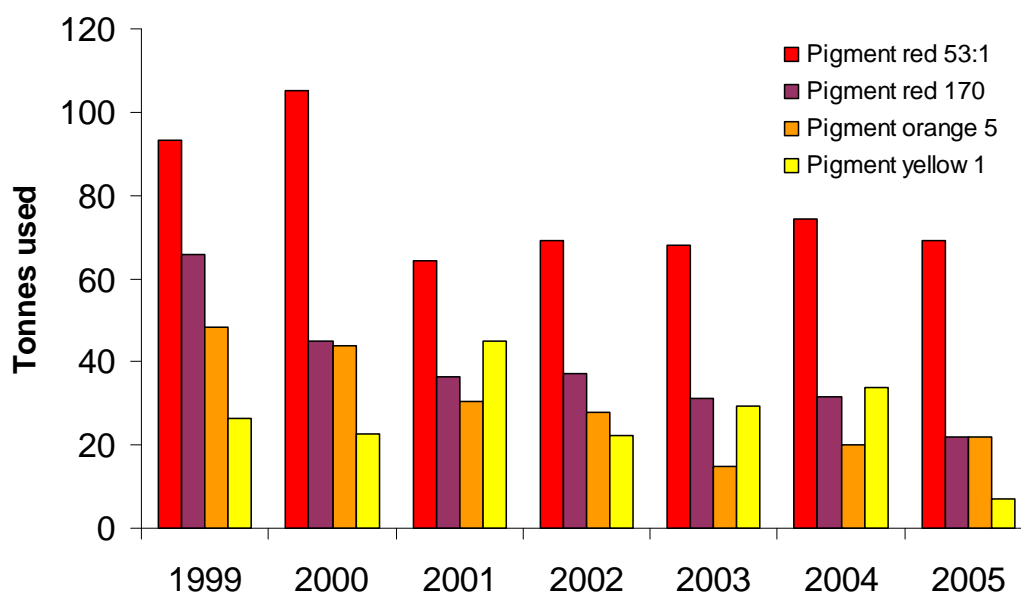


Figure 1. The Swedish use of the pigments included in the screening between the years 1999 and 2005 (Spin, 2008).

The pigments are used as colouring and reprographic agents, in paints, lacquers and varnishes. Use categories found in the Spin database are presented in Table 6.

Table 6. Use categories for the pigments 2005 (Spin, 2008).

Pigment	Use category
Pigment yellow 1	Colouring agents
Pigment orange 5	Colouring agents, Reprographic agents, Paints, lacquers and varnishes
Pigment red 53:1	Colouring agents, Reprographic agents
Pigment red 170	Colouring agents, Reprographic agents, Paints, lacquers and varnishes

Emissions of pigments to the environment can be expected to occur during production of materials containing the pigments, through losses during products use, and during disposal and recycling of materials containing the pigments. According to the OECD SIDS Initial Assessment report (OECD SIDS, 2002), main releases of Pigment red 53:1 are expected during paper recycling.

4 Previous measurements in the environment

No previous measurements of the pigments in the environment have been found during the literature search. However, for Pigment red 53:1, a concentration of 3.4 µg/l in effluent water from a German deinking plant has been reported (OECD SIDS, 2002).

5 Sampling strategy and study sites

A sampling strategy was developed in order to determine concentrations of the pigments in different matrices in the Swedish environment. Due to the lack of previous measurements in the environment, the sampling programme was based on use and the identified possible emission sources and the predicted environmental distribution. The sampling programme is summarised in Table 7, and details of the samples are given in the Appendix 1, Table A 1.

Table 7. Samples included in the screening programme.

Site	STP waters	Sludge	Storm/Leachate waters	Surface water	Sediment	Soil	Fish	Total
Background								
Gårdsjön						1		1
National background lakes				3	3		3	9
Diffuse sources								
Municipal STPs	8	6		1	2			17
Stockholm			3	3	3		3	12
Point sources								
Landfills		1	3					4
Paper mill	2	3						5
Public laundries	2							2
Marina						1		1
Total	12	10	6	7	8	2	6	51

Due to the low water solubilities of the pigments, solid matrices such as sediments, soil, sludge, and the particulate phases of storm and leachate waters, were considered important matrices. In background areas, sediment, soil, fish and surface waters were sampled. The screening for diffuse sources included STP waters and sludge. From the urban environment storm waters, surface waters, sediments and fish were collected. The samples from identified point sources included leachate waters and sludge from landfills, process waters and sludge from the deinking process of recycled paper in a paper mill, and effluent waters from public laundries. A soil sample from a marina was also taken after the spring time preparations and launching. There were difficulties in obtaining some of the samples, e.g. leachate water sludge from landfills and effluent waters from public laundries. This was compensated by additional sampling based on the preliminary results. Complementing samples included influent and effluent water from the Gässlösa STP, and sediments and surface water from the recipient river Viskan.

6 Methods

6.1 Sampling

The staff at the different sewage treatment plants (STPs) collected **sludge** samples from the anaerobic chambers. The sludge was transferred into glass jars and stored in a freezer (-18°C) until analysed. **Influent** and **effluent waters** were sampled in 1l glass bottles.

Surface **sediment** (0-2 cm) samples were collected by means of a Kajak sampler. The sediment was transferred into muffled (400°C) glass jars and stored in a freezer (-18°C) until analysed. Three sediment samples from the national background lakes were provided by the Swedish Museum of Natural History.

Fish were collected by means of fishing net. The net fishing was approved by the fishery authorities in Stockholm and the ethical board for animal testing in northern Stockholm (D. no. 572/07). Perch (*Perca fluviatilis*) representing the second-fifth year classes were selected for analysis.

The fish were individually wrapped in cleaned aluminium foil and stored in a freezer (-18°C) until analysed. Fish muscle was dissected for analysis by means of solvent washed scalpels. Samples of dissected fish muscle from three national background lakes were provided from the specimen bank at the Swedish Museum of Natural History. These samples were stored at -18°C in pre-cleaned glass jars.

Surface water samples from background lakes and from the city of Stockholm were collected in glass bottles.

Storm water samples collected during periods of sufficient precipitation.

Leachate water and **leachate water sludge** samples were collected in 1l glass bottles and glass jars.

A pooled surface **soil** (0-2 cm) sample from a background area was collected in a glass jar.

Surface **Soil** (0-2 cm) from a marina in the City of Stockholm was collected in a plastic bag.

Process water and **process water sludge** were collected from the deinking plant in a paper mill in 1l glass bottles and glass jars.

6.2 Analysis

6.2.1 Method development

The pigments are practically insoluble in water and in most organic solvents. In order to make stock solutions of the individual pigments, a wide range of solvents has been considered. Pigment yellow 1 (PY1), Pigment orange 5 (PO5) and Pigment red 53:1 (PR53) were readily soluble in dimethylformamide, and stock solutions were made in the concentration range 64 – 156 ng/μL. For Pigment red (PR170) and Pigment violet 23 (PV23), stock solutions of 10-20 ng/μL in ethyl acetate were made. For Pigment blue 15 (PB15) and Pigment green 7 (PG7), both of them phthalocyanine compounds, toluene was the most efficient solvent, and stock solutions were made at 7 and 17 ng/μL, respectively. Further dilutions of the stock solutions were made in acetonitrile.

The instrumental analysis was performed by liquid chromatography-mass spectrometry (LC-MS) (see instrumentation below). Two ionization modes have been considered: Electrospray (ES) ionization and atmospheric pressure chemical ionization (APCI). Ionization experiments were performed by direct infusion of the compounds into the ion source. PY1, PO5 and the two red pigments were readily ionized in negative mode ES and negative mode APCI. For the remaining three pigments - PV23, PB15 and PG7 - no ionization was achieved in either of the ionization modes. Due to this lack of successful instrumental analysis, these three pigments have been omitted from the method. The development of the analytical methods is further described in Annex II.

6.2.2 Water samples

In water samples, the pigments may be adsorbed to particles due to their low water solubility. To analyse the total amount of pigments the samples were filtered on glass fibre (A/E) filters, and the liquid and solid phases analysed separately.

After filtration, the liquid phase of the sample was applied to a polymeric solid phase extraction (SPE) column. The columns were rinsed to remove some of the organic matrix compounds, and finally eluted with a mixture of organic solvents.

The particulate phase of the water samples was extracted by accelerated solvent extraction (ASE). The extraction was performed using a polar organic solvent at an elevated temperature and pressure.

Both the SPE and ASE eluents were injected into the liquid chromatography time of flight mass spectrometry (LC-TOF-MS) (see instrumentation) without further concentration or clean-up.

6.2.3 Sediment, sludge, soil and fish samples

For sediments and biota, extractions were performed by ASE as described for the particulate matter in water samples.

6.2.4 Quality control

Due to the lack of a commercially available internal standard suitable for the analysis, the quantitation was performed using external, matrix-matched standard calibration. For quantitation of water samples, a pooled sample of STP influent not containing the analytes was used for matrix-matched standards. An aliquot of the pooled sample was filtered and processed as described previously. The ASE and SPE eluents from this sample was used to dilute stock solutions of the analytes, making standard solutions used for quantitation.

During method development, recovery was monitored by extracting spiked samples of tap water and STP influent. For method validation, five samples of pooled STP influent not containing the analytes were spiked and used as QC samples. The compounds were added to both liquid and particle phase after filtration, in a concentration of approximately 0.4 µg/L.

6.2.5 Instrumentation

The instrumental analysis was performed on a Waters LCT Premier XE Time-Of-Flight mass spectrometer coupled to a Waters Acquity UPLC high pressure liquid chromatograph. Chromatographic separation was achieved on a 10 cm, 2.1 mm i.d., 1.7 µm particle size Waters Acquity phenyl column using a water/acetonitrile mobile phase gradient. The compounds were detected using the [M-H]⁻ species generated in negative electrospray mode. Pigment Red 53:1, a barium salt with the formula (C₁₇H₁₂ClN₂O₄S)₂*Ba, was determined as the free sulfonate.

7 Results and discussion

All the results from the measurements of the pigments included in the screening can be found in Table A 2 and Table A 3 in Appendix 1.

7.1 Background areas

Neither of the pigments analysed were found in soil, surface water, sediment or fish samples from the background areas. Detection limits for the pigments in the different matrices in samples from the background areas are presented in Table 8.

Table 8. Detection limits for the analysed pigments in samples from background areas.

	Unit	Pigment yellow 1	Pigment orange 5	Pigment red 53:1	Pigment red 170
Soil	ng/g dw	< 59	< 11	< 5.4	< 137
Surface water	ng/l	< 9.5 - < 15	< 1.1 - < 2.3	< 3.9 - < 5.0	< 48 - < 85
Sediment	ng/g dw	< 21 - < 77	< 5.9 - < 8.9	< 0.7 - < 4.4	< 23 - < 69
Fish	ng/g ww	< 13 - < 21	< 1.1 - < 4.2	< 0.9 - < 1.2	< 29 - < 92

7.2 Diffuse sources

7.2.1 Sewage treatment plants

The concentrations of pigments found in sludge and in influent and effluent waters from STPs are presented in Figure 2 and in Figure 3.

Three of the pigments, Pigment Red 170, Pigment Red 53:1 and Pigment Yellow 1, were detected in sludge from the municipal sewage treatment plants, see Figure 2. Highest concentrations, 100000 ng/g dw and 88000 ng/g dw of Pigment Red 170, were found in the two sludge samples taken from Gässlösa STP at two consecutive months. These concentrations are approximately 1000 times higher compared to the levels found in sludge from the other STPs. The highest concentrations of the other detected pigments were also found in sludge from this STP. This STP receives effluents from industries using pigments.

Due to the high concentrations detected in sludge from the Gässlösa STP, the screening programme was complemented with additional samples in August 2008. These samples consisted of influent and effluent waters from the STP, sediment upstream and sediment and surface water downstream in the recipient river Viskan.

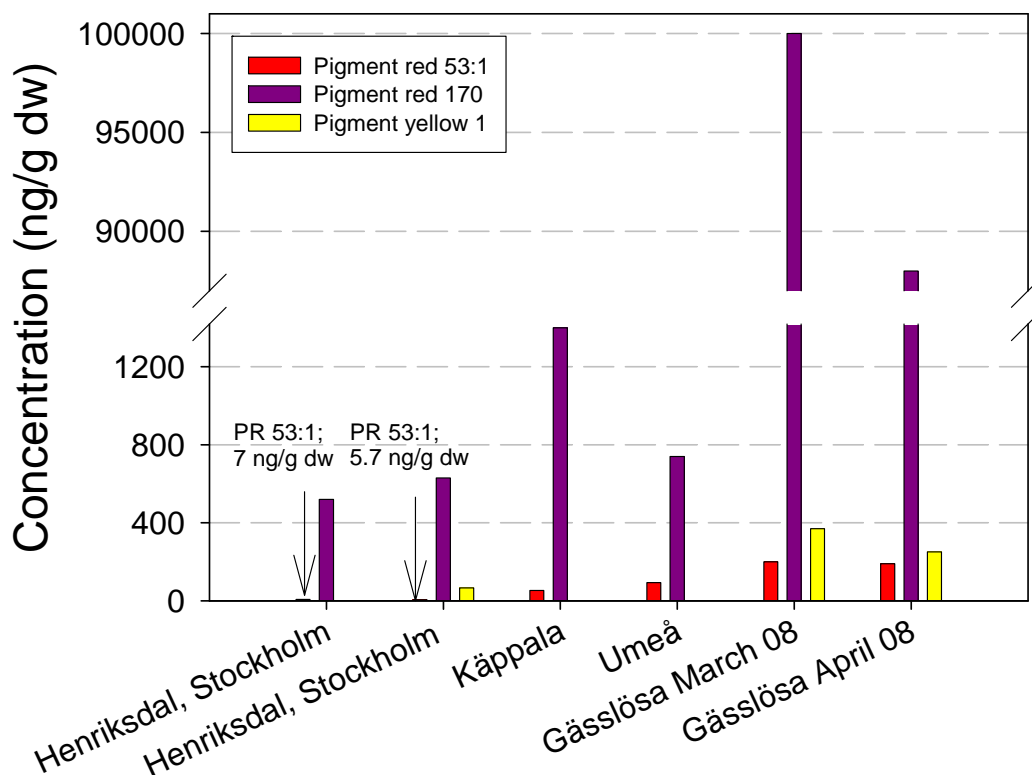


Figure 2. Concentrations of pigments in sludge from STPs.

All of the pigments included in the screening were found in STP influent waters, see Figure 3. Pigment yellow 1 was found in the highest concentrations. It was found in influent waters from three of the STPs in the range 130-380 ng/l. Pigment red 53:1 was found in all four influent waters in the range 7-27 ng/l. Pigment red 170 was found in effluent waters from three of the STPs in the range 68-90 ng/l, whereas Pigment orange 5 was found in two out of four influent water samples at the concentrations 27 and 67 ng/l.

For Pigment yellow 1, red 170 and red 53:1, 100% of the content in influent water were found in the particulate phase. For Pigment orange 5 a small fraction, 7.5 % of the content, was found in the water phase in the influent sample from the Henriksdal STP.

The results from the Gässlösa STP differed from the other STPs. For this STP only Pigment red 53:1 could be found in the influent water. This pigment was also found in the effluent water at approximately the same concentration but with 38% of the content present in the water phase. The water samples from the Gässlösa STP were taken in August 2008, thus not during the same period as the other samples. Since the highest concentrations of pigments in sludge were measured in samples from this STP, relatively high concentrations were also expected to be found in the influent water, but this was not the case. The discrepancy between this STP and the other ones, and between measured levels and expected, may thus be due to variation in load with time.

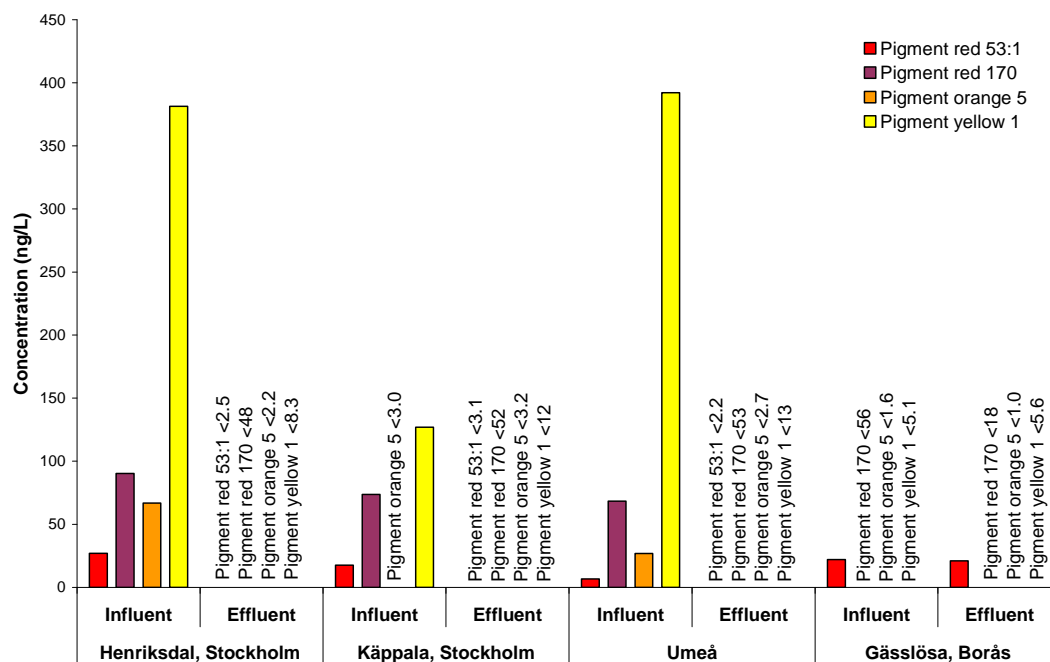


Figure 3. Concentrations of pigments in influent and effluent waters from STPs.

The lack of pigments in effluent waters from three of the STPs indicates that removal of the pigments take place during the waste water treatment process.

The results from the measurements in surface water and sediments of the recipient Viskan are presented in Table 9. None of the pigments were detected in the surface water sample, but both Pigment red 53:1 and Pigment red 170 were found in the sediment samples. Concentrations were slightly higher upstream compared to downstream of the effluent outlet, thus emissions to this recipient can not only be attributed to the STP.

Table 9. Pigment concentrations in surface water and sediments of Viskan, recipient of the Gässlösa STP.

Sample	Unit	Pigment red 53:1	Pigment red 170	Pigment orange 5	Pigment yellow 1
Surface water downstream	ng/l	<1.8	<27	<1.1	<2.5
Sediment downstream	ng/g dw	1.4	65	<2.0	<0.7
Sediment upstream	ng/g dw	3.9	96	<2.0	<0.7

7.2.2 Urban environment

To study diffuse sources in the urban environment, surface waters, sediments, fish and storm waters were sampled in the urban area of Stockholm. In surface water only Pigment red 53:1 was found, at a concentration of 5 ng/l in the sample from Riddarfjärden, see Table 10. In sediments both Pigment red 53:1 and Pigment red 170 were found. Pigment red 53:1 was found in all three analysed samples, whereas Pigment red 170 was found in the sediment samples from Årstaviken and Riddarfjärden. Neither of the pigments was found in the fish samples.

Table 10. Concentrations of pigments in surface waters, sediments and fish from the city of Stockholm.

Sampling site	Sample type	Unit	Pigment yellow 1	Pigment orange 5	Pigment red 53:1	Pigment red 170
Stora Essingen	Surface water	ng/l	< 12	< 2.9	< 3.9	< 85
	Sediment	ng/g dw	< 52	< 8.0	7.6	< 110
	Fish	ng/g ww	< 16	< 3.3	< 1.5	< 88
Årstaviken	Surface water	ng/l	< 10	< 2.4	< 4.1	< 71
	Sediment	ng/g dw	< 29	< 8.5	5.9	190
	Fish	ng/g ww	< 20	< 4.9	< 1.1	< 10
Riddarfjärden	Surface water	ng/l	< 20	< 2.1	5	< 69
	Sediment	ng/g dw	< 21	< 3.4	13	330
	Fish	ng/g ww	< 18	< 2.8	< 1.0	< 80

All of the determined pigments were found in storm waters from the city of Stockholm, see Table 11. Concentrations were below the detection limits in the sample from Eugeniattunneln whereas high concentrations, in the same range or higher than in STP influent waters, were found in the samples from Torstenssonsgatan and Sveavägen. In these samples, 100% of the detected pigments were found in the particulate phase.

Table 11. Concentrations of pigments in storm water samples from the city of Stockholm

Sampling site	Unit	Pigment yellow 1	Pigment orange 5	Pigment red 53:1	Pigment red 170
Eugeniattunneln	ng/l	< 17	< 1.8	< 4.0	< 77
Torstenssonsgatan	ng/l	750	65	210	390
Sveavägen	ng/l	< 68	59	370	2800

7.3 Point sources

Leachate waters and sludge from landfills were analysed to study the potential leachage of pigments from products during waste disposal. Pigments were not found in any of the landfill related samples, see Table 12.

Table 12. Concentrations of pigments in leachate waters and sludge from landfills.

Site	Matrix	Unit	Pigment yellow 1	Pigment orange 5	Pigment red 53:1	Pigment red 170
Högbytorp landfill	Leachate water	ng/l	< 12	< 2.1	< 3.8	< 39
	Sludge	ng/g ww	< 6.3	< 1.3	< 0.2	< 15
Kovik landfill	Leachate water	ng/l	< 16	< 5.4	< 5.4	< 76
Löt landfill	Leachate water	ng/l	< 11	< 3.2	< 4.0	< 65

Since the pigments are used as colouring agents and textiles often are heavily dyed, effluent waters from public laundries were sampled to study the potential emissions of the pigments from textiles. All four pigments could be detected in effluent water from one of the public laundries, see Table 13. This sample will be treated in a municipal sewage treatment plant. In effluent water from the second laundry, only Pigment red 53:1 was found. This effluent water was sampled after the passage through a municipal sewage treatment plant. Interestingly most of the Pigment red 53:1 detected in the two samples was found in the water phase, 85 % and 100% respectively. This is opposite to the distribution in the STP influent waters where 100% of the pigment was found in the particulate phase, but similar to effluent water from Gässlösa STP where 38% was found in the

water phase. The other pigments were only detected in the particulate phase of the laundry effluent samples.

Table 13. Concentrations of pigments in effluent waters from public laundries.

Public laundry	Unit	Pigment yellow 1	Pigment orange 5	Pigment red 53:1	Pigment red 170
Göteborg ^a	ng/l	190	98	85	1000
Svealand ^b	ng/l	< 12	< 2.4	13	< 32

^a sample taken prior to treatment in a municipal STP

^b sample taken after treatment in a municipal STP

Since spring preparations of boats, including sanding, painting and polishing, often take place in the open air resulting in the spread of dust containing pigments, a soil sample from a marina was analysed. Concentrations of 130 ng/kg dw and 6.8 ng/kg dw were found for Pigment red 170 and Pigment orange 5 respectively. Concentrations of Pigment red 53:1 and Pigment yellow 1 were below the detection limits.

High concentrations of all four analysed pigments were found in process water and sludge from the deinking of recycled paper, from a paper mill, see Table 14.

Table 14. Concentrations of pigments in process waters and sludge from the deinking of recycled paper in a paper mill.

	Unit	Pigment yellow 1	Pigment orange 5	Pigment red 53:1	Pigment red 170
Process water stage 1	ng/l	61000	9800	450000	8900
Process water stage 2	ng/l	27000	3200	160000	6000
Sludge 1	ng/g dw	800	650	4200	1600
Sludge 2	ng/g dw	370	< 6.5	1100	420
Inj to floatation step	ng/g dw	< 142	< 41	1500	< 361

8 Conclusions

Development of analytical methods was successful for the pigments Pigment red 53:1, Pigment red 170, Pigment yellow 1 and Pigment orange 5. Analytical methods for the pigments Pigment violet 23, Pigment blue 15 and Pigment green 7 could not be established and they were thus excluded from the screening.

All four analysed pigments could be found in influent waters from municipal STPs, but only Pigment red 53:1 could be detected in effluent water. Three of the pigments, Pigment red 53:1, Pigment red 170 and Pigment yellow 1, were found in sludge samples. The relative distribution of the pigments differed in influent water and sludge samples.

Pigments are removed in the sewage treatment process, one mechanism probably being adsorption to sludge. This is indicated by the partitioning of the pigments to the particulate phase in the influent waters, and the content found in sludge from municipal STPs.

All four pigments could also be found in storm water samples from the city of Stockholm.

Three of the pigments, Pigment red 53:1, Pigment red 170 and pigment orange 5, could be found in recipient samples from the urban environment. Pigment red 53:1 and Pigment red 170 were found

in sediment samples from the city of Stockholm and in sediment samples from the recipient Viskan of the Gässlösa STP. Pigment red 53:1 was also found in one surface water sample from the city of Stockholm. Pigment red 170 and Pigment orange 5 could be found in the soil sample from a Marina.

All four pigments could be found in samples from different stages in the process of deinking of recycled paper and also in effluent water from a public laundry. This indicates that paper and textiles may be sources to emissions of all four pigments.

All together, the presence of the pigments in STP sludge and waters, in effluent waters from public laundries, in storm waters, in sediments, in surface water and in soil, indicate that diffuse emissions of the pigments occur.

None of the pigments could be detected in the samples from background areas. Emissions in and transport to rural areas thus seems to be minor.

9 Acknowledgements

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Appendix 1: Information on samples and list of results

Table A 1. List of samples for analysis of pigments.

Sample ID	Category	Matrix	Site	Sampling date	Comments	Coordinates
6036	Background	Soil	Gårdsjön	2007-09-13		
6223	Background	Surface water	Bysjön, Årjäng	2007-09-06		N658086 E130264
6224	Background	Surface water	Spjutsjön, Falun	2007-09-03		N672467 E148031
6225	Background	Surface water	Gipsjön, Malung	2007-09-04		N672729 E138082
6226	Background	Sediment	Bysjön, Årjäng	2007-09-06		N658086 E130264
6227	Background	Sediment	Spjutsjön, Falun	2007-09-03		N672467 E148031
6228	Background	Sediment	Gipsjön, Malung	2007-09-4		N672729 E138082
6872	Background	Fish	Bysjön, Årjäng	2007-09-06	Perch	N658086 E130264
6873	Background	Fish	Gipsjön, Malung	2007-09-04	Perch	N672729 E138082
6874	Background	Fish	Spjutsjön, Falun	2007-09-03	Perch	N672467 E148031
6559	Diffuse, STP	Influent	Henriksdal, Stockholm	2007-10-25		
6565	Diffuse, STP	Effluent	Henriksdal, Stockholm	2007-10-25		
6584	Diffuse, STP	Sludge	Henriksdal, Stockholm	2007-10-25		
6035	Diffuse, STP	Sludge	Henriksdal, Stockholm	2007-09		
6766	Diffuse, STP	Influent	Käppala, Stockholm	2007-12-05		
6767	Diffuse, STP	Effluent	Käppala, Stockholm	2007-12-06		
6844	Diffuse, STP	Sludge	Käppala, Stockholm	2007-12-20		
6891	Diffuse, STP	Influent	Umeå	2008-03-27		
6892	Diffuse, STP	Effluent	Umeå	2008-03-27		
6896	Diffuse, STP	Sludge	Umeå	2008-04-02		
7000	Diffuse, STP	Influent	Gässlösa, Borås	2008-08-06		
7001	Diffuse, STP	Effluent	Gässlösa, Borås	2008-08-06		
6909	Diffuse, STP	Sludge	Gässlösa, Borås	2008-03		
6936	Diffuse, STP	Sludge	Gässlösa, Borås	2008-04-18		
7004	Diffuse	Surface water	Viskan, downstream Gässlösa STP	2008-08-05		
7002	Diffuse	Sediment	Viskan, upstream Gässlösa STP	2008-08-05		

Sample ID	Category	Matrix	Site	Sampling date	Comments	Coordinates
7003	Diffuse	Sediment	Viskan, downstream Gässlösa STP	2008-08-05		
6364	Diffuse, urban	Sediment	Stockholm Riddarfjärden	2007-10-03		N6580141 E1627276
6357	Diffuse, urban	Surface water	Stockholm Stora Essingen	2007-10-03		N6579241 E1623643
6358	Diffuse, urban	Sediment	Stockholm Stora Essingen	2007-10-03		N6579241 E1623643
6359	Diffuse, urban	Fish	Stockholm Stora Essingen	2007-10-03	Perch	N6579241 E1623643
6360	Diffuse, urban	Surface water	Stockholm Årstaviken	2007-10-03		N6578147 E1628330
6361	Diffuse, urban	Sediment	Stockholm Årstaviken	2007-10-03		N6578147 E1628330
6362	Diffuse, urban	Fish	Stockholm Årstaviken	2007-10-03	Perch	N6578147 E1628330
6363	Diffuse, urban	Surface water	Stockholm Riddarfjärden	2007-10-03		N6580141 E1627276
6365	Diffuse, urban	Fish	Stockholm Riddarfjärden	2007-10-03	Perch	N6580141 E1627276
6616	Diffuse, urban	Storm water	Stockholm Eugeniattunneln nov 07	2007-11-07		N6583266 E1627181
6803	Diffuse, urban	Storm water	Stockholm, Torstenssonsgatan	2007-12-13		N6581298 E1629972
6805	Diffuse, urban	Storm water	Stockholm Sveavägen	2007-12-13		N6582795 E1627895
6715	Point source	Effluent	Public laundry southern Götaland	2007-11-21		
6750	Point source	Effluent	Public laundry eastern Svealand	2007-11		
6398	Point source	Leachate water	Landfill Löt	2007-10-09		N6615315 E1641725
6540	Point source	Leachate water	Landfill Högbytorp	2007-10-23		N6604110 E1603252
6460	Point source	Leachate water	Landfill Kovik	2007-10-15		N6583454 E1645162
6893	Point source	Leachate water sludge	Landfill Högbytorp	2007-10-23		N6604110 E1603252
6917	Point source	Process water	Paper mill Svartslam 1	2008-04-15		
6918	Point source	Process water	Paper mill. Svartslam 2	2008-04-15		
6920	Point source	Process sludge	Paper mill Avvattnat fiber o svartslam	2008-04-15		
6919	Point source	Sludge	Paper mill svartslam+fiberslam	2008-04-15		
6921	Point source	Sludge	Paper mill injekt till flotationssteg	2008-04-15		
6938	Point source	Soil	Stockholm, Årstavikens Segelsällskap	2008-05-07		N 59 18 15.30; E 18 4 8.64

Table A 2. Concentrations of pigments in analysed water samples.

Sample ID	Matrix	Category	Site	Particulate phase, ng/L				Water phase, ng/L				Total, ng/L				Part. cont. g/l
				PR53:1	PR170	PO5	PY1	PR53:1	PR170	PO5	PY1	PR53:1	PR170	PO5	PY1	
6223	Surface water	Background	Bysjön, Årjäng	<2.5	<28	<7.5	<52	<4.4	<48	<1.2	<9.5	<4.4	<48	<1.2	<9.5	0.02
6224	Surface water	Background	Spjutsjön, Falun	<3.8	<32	<15	<97	<3.9	<85	<2.3	<15	<3.9	<85	<2.3	<15	-
6225	Surface water	Background	Gipsjön, Malung	<2.6	<30	<20	<67	<5.0	<77	<2.3	<14	<5.0	<77	<2.3	<14	0.08
6559	Influent	Diffuse, STP	Henriksdal, Stockholm	27	90	62	380	<3.3	<76	5	<15	27	90	67	380	0.77
6565	Effluent	Diffuse, STP	Henriksdal, Stockholm	<2.6	<23	<3.2	<12	<2.5	<48	<2.2	<8.3	<2.5	<48	<2.2	<8.3	0.37
6766	Influent	Diffuse, STP	Käppala, Stockholm	18	74	<8.5	130	<3.0	<38	<3.0	<18	18	74	<3.0	130	0.53
6767	Effluent	Diffuse, STP	Käppala, Stockholm	<3.3	<30	<13	<69	<3.1	<52	<3.2	<12	<3.1	<52	<3.2	<12	0.41
6891	Influent	Diffuse, STP	Umeå	7	68	27	390	<5.1	<58	<2.1	<13	7	68	27	390	0.73
6892	Effluent	Diffuse, STP	Umeå	<2.5	<30	<8.4	<60	<2.2	<53	<2.7	<13	<2.2	<53	<2.7	<13	0.26
7000	Influent	Diffuse, STP	Gässlösa, Borås	22	<11	<2.6	<8.0	<0.8	<56	<1.6	<5.1	22	<56	<1.6	<5.1	0.33
7001	Effluent	Diffuse, STP	Gässlösa, Borås	13	<11	<3.2	<12	8.0	<18	<1.0	<5.6	21	<18	<1.0	<5.6	0.22
7004	Surface water	Diffuse	Viskan	n.a.	n.a.	n.a.	n.a.	<1.8	<27	<1.1	<2.5	<1.8	<27	<1.1	<2.5	-
6357	Surface water	Diffuse, urban	Stockholm Stora Essingen	<4.8	<35	<16	<10	<3.9	<85	<2.9	<12	<3.9	<85	<2.9	<12	0.71
6360	Surface water	Diffuse, urban	Stockholm Årstaviken	<1.4	<44	<9.6	<18	<4.1	<71	<2.4	<10	<4.1	<71	<2.4	<10	0.9
6363	Surface water	Diffuse, urban	Stockholm Riddarfjärden	5	<23	<13	<67	<2.5	<69	<2.1	<20	5	<69	<2.1	<20	0.77
6616	Storm water	Diffuse, urban	Stockholm Eugeniatunneln	<3.7	<18	<12	<44	<4.0	<77	<1.8	<17	<4.0	<77	<1.8	<17	0.84
6803	Storm water	Diffuse, urban	Stockholm Torstenssonsgatan	210	390	65	750	<6.5	<120	<8.3	<98	210	390	65	750	3.27
6805	Storm water	Diffuse, urban	Stockholm Sveavägen	370	2800	59	<133	<2.3	<124	<7.1	<68	370	2800	59	<68	9.7
6398	Leachate water	Point source	Land fill Löt	<1.9	<30	<19	<49	<4.0	<65	<3.2	<11	<4.0	<65	<3.2	<11	1.3
6460	Leachate water	Point source	Land fill Kovik	<2.0	<35	<20	<65	<5.4	<76	<5.4	<16	<5.4	<76	<5.4	<16	3.99
6540	Leachate water	Point source	Land fill Högbytorp	<5.1	<18	<28	<91	<3.8	<39	<2.1	<12	<3.8	<39	<2.1	<12	6.52
6715	Effluent	Point source	Public laundry southern Götaland	13	1000	98	190	72	<70	<2.0	<9.4	85	1000	98	190	0.91
6750	Effluent	Point source	Public laundry Svealand	<1.8	<18	<24	<56	13	<32	<2.4	<12	13	<32	<2.4	<12	2.63
6917	Process water	Point source	Paper mill Svartslam 1	430000	8900	9800	61000	21000	<11	<2.3	<34	450000	8900	9800	61000	80
6918	Process water	Point source	Paper mill Svartslam 2	150000	6000	3200	27000	5100	<12	<3.6	<8.7	160000	6000	3200	27000	108

Table A 3. Concentrations of pigments in analysed solid matrices.

Sample ID	Matrix	Category	Site	Unit	PR53:1	PR170	PO5	PY1
6872	Fish	Background	Bysjön, Årjäng	ng/g ww	<0.9	<29	<4.2	<14
6873	Fish	Background	Gipsjön, Malung	ng/g ww	<1.2	<45	<1.1	<21
6874	Fish	Background	Spjutsjön, Falun	ng/g ww	<1.1	<92	<1.4	<13
6226	Sediment	Background	Bysjön, Årjäng	ng/g dw	<0.7	<23	<5.9	<21
6227	Sediment	Background	Spjutsjön, Falun	ng/g dw	<1.3	<63	<8.9	<56
6228	Sediment	Background	Gipsjön, Malung	ng/g dw	<4.4	<69	<7.4	<77
6036	Soil	Background	Gärdsjön	ng/g dw	<5.4	<137	<11	<59
6035	Sludge	Diffuse, STP	Henriksdal, Stockholm	ng/g dw	7.0	520	<2.0	<14
6584	Sludge	Diffuse, STP	Henriksdal, Stockholm	ng/g dw	5.7	630	<1.6	66
6844	Sludge	Diffuse, STP	Käppala, Stockholm	ng/g dw	53	1400	<5.4	<25
6896	Sludge	Diffuse, STP	Umeå	ng/g dw	93	740	<4.8	<38
6909	Sludge	Diffuse, STP	Gässlösa, Borås	ng/g dw	200	100000	<4.5	370
6936	Sludge	Diffuse, STP	Gässlösa, Borås	ng/g dw	190	88000	<4.4	250
6359	Fish	Diffuse, urban	Stockholm Stora Essingen	ng/g ww	<1.5	<88	<3.3	<16
6362	Fish	Diffuse, urban	Stockholm Årstaviken	ng/g ww	<1.1	<10	<4.9	<20
6365	Fish	Diffuse, urban	Stockholm Riddarfjärden	ng/g ww	<1.0	<80	<2.8	<18
6358	Sediment	Diffuse, urban	Stockholm Stora Essinge	ng/g dw	7.6	<110	<8.0	<52
6361	Sediment	Diffuse, urban	Stockholm Årstaviken	ng/g dw	5.9	190	<8.5	<29
6364	Sediment	Diffuse, urban	Stockholm Riddarfjärden	ng/g dw	13	330	<3.4	<21
6893*	Leachate water sludge	Point source	Landfill Högbytorp	ng/g ww	<0.2	<15	<1.3	<6.3
6920	Process sludge	Point source	Paper mill. Avvattnat fiber o svartaslam	ng/g dw	1100	420	<6.5	370
6919	Sludge	Point source	Paper mill svartslam+fiberslam	ng/g dw	4200	1600	650	800
6921	Sludge	Point source	Paper mill injekt till flotationssteg	ng/g dw	1500	<361	<41	<142
6938	Soil	Point source	Stockholm, Årstavikens segelsällskap	ng/g dw	<0.3	130	6.8	<0.6
7002	Sediment	Diffuse	Viskan	ng/g dw	3.9	96	<2.0	<0.7
7003	Sediment	Diffuse	Viskan	ng/g dw	1.4	65	<2.0	<0.7

Appendix 2: Development of analytical methods

Determination of pigments in environmental samples

Seven organic pigments were selected for determination in environmental samples. The compounds of interest were Pigment Yellow 1 (PY1), Pigment Orange 5 (PO5), Pigment Red 53:1 (PR53), Pigment Red 170 (PR170), Pigment Violet 23 (PV23), Pigment Blue 15 (PB15) and Pigment Green 7 (PG7). The aim was to develop a method based on liquid chromatography-Time-of-Flight mass spectrometry (LC-TOF-MS). During method development, three of the compounds were omitted, as they were not amenable to LC-TOF-MS.

Standard solutions

The pigments are practically insoluble in water and most organic solvents. In order to make stock solutions of the individual pigments, a wide range of solvents has been considered. PY1, PO5 and PR53 were readily soluble in dimethylformamide, and stock solutions were made in the concentration range 64 – 156 ng/ μ L. For PR170 and PV23, stock solutions of 10-20 ng/ μ L in ethyl acetate were made. PB15 and PG7, both phtalocyanine compounds, were partially soluble in toluene, and stock solutions were made at 7 and 17 ng/ μ L, respectively. Further dilutions of the stock solutions were made in acetonitrile. The solubilities of the pigments are presented in Table 1.

Table 1. Solubility of pigments in various solvents.

	Methanol	DMF	DMSO	Ethyl acetate	Toluene	AcCN	DCM	Acetone
PY1	-	+						
PO5	-	+						
PR53:1	-	+						
PR170	-	-	-	+	-	(+)	(+)	-
PV23	-	-	-	+	-	(+)	(+)	-
PB15	-	-	-	-	(+)	-	-	-
PG7	-	-	-	-	(+)	-	-	-

LC-MS analysis

Method development:

Two ionization modes were considered: Electrospray (ES) ionization and atmospheric pressure chemical ionization (APCI). Ionization experiments were performed by direct infusion of single-compound solutions into the ion source. PY1, PO5 and the two red pigments were readily ionized in negative mode ES and negative mode APCI. For the remaining three pigments - PV23, PB15 and PG7 - no ionization was achieved in either of the ionization modes, probably due to a lack of readily ionizable groups in the molecular structures of these compounds. As the instrumental analysis for these three compounds was unsuccessful, they were omitted from the final method.

Analysis of four pigments:

The instrumental analysis was performed on a Waters LCT Premier XE Time-Of-Flight mass spectrometer coupled to a Waters Acquity UPLC high pressure liquid chromatograph. Chromatographic separation was achieved on a 10 cm, 2.1 mm i.d., 1.7 μ m particle size Waters Acquity phenyl column using a water/acetonitrile mobile phase gradient. The compounds were detected using the [M-H]⁻ species generated in negative electrospray mode. Pigment Red 53:1, a barium salt with the formula (C₁₇H₁₂ClN₂O₄S)₂*Ba, was determined as the free sulfonate.

Sample preparation

Analysis of water samples:

In water samples, the pigments may be adsorbed to particles due to their low water solubility. To analyse the total amount of pigments the samples were filtered using glass fibre (A/E) filters, pore size 1 µm, and the liquid and solid phases analysed separately.

After filtration, the liquid phase of the sample was acidified and applied to a Strata-X solid phase extraction (SPE) column. The columns were rinsed with 45% methanol in water to remove some of the organic matrix compounds, and finally eluted with ethyl acetate:methanol 1:1.

The particulate phase of the water samples was extracted by accelerated solvent extraction (ASE). The extraction was performed using acetonitrile at an elevated temperature and pressure. The 22 mL ASE cells were packed using three filters, approximately 1 g of diatomaceous earth (DE), the folded sample filter and finally filled up with DE.

Both the SPE and ASE eluents were injected into the LC-TOF-MS without further concentration, as concentrating the samples was shown to generate a severe loss in recovery, probably due to adsorption to the sample vessel.

Sediment, sludge and biota:

For sediments, sludge and biota, extraction was performed by ASE as described for the particulate matter in water samples. Approximately 2 g of sediment or 1 g of homogenised fish was mixed with DE and packed in 11 mL ASE cells. The sediment and sludge samples were not dried prior to extraction. Water content of the samples was determined in separate aliquots of the samples.

Quantitation

Due to the lack of a commercially available internal standard suitable for the analysis, the quantitation was performed using external, matrix-matched standard calibration. For quantitation of water samples, a pooled sample of STP influent not containing the analytes was used for matrix-matched standards. An aliquot of the pooled sample was filtered and processed as described previously. The ASE and SPE eluents from this sample was used to dilute stock solutions of the analytes, making standard solutions used for quantitation. For quantitation of fish samples, the standard was made up in a blank extract of perch muscle. For sediment and sludge, an extract of a Barents Sea sediment was used to dilute the standard used for calibration.

Method validation

Quality control samples - water

For method validation, five samples of pooled STP influent not containing the analytes were spiked and used as QC samples. The compounds were added to both liquid and particle phase after filtration, in a concentration of approximately 400 ng/L. In addition, one aliquot of the pooled sample was used as a procedural blank.

Quality control samples - sediment

To monitor recovery from sediment and sludge samples, five replicate samples of a Barents Sea sediment were spiked to approximately 25 ng/g wet weight and analysed as previously described.

Quality control samples – fish

Five replicate samples of homogenised perch muscle were spiked to approximately 200 ng/g wet weight and analysed with the samples.

Validation results

Compound	Water samples				Sediment		Fish	
	Liquid		Solid		Recovery, %	RSD, %	Recovery, %	RSD, %
	Recovery, %	RSD, %	Recovery, %	RSD, %				
PR53	60	22	79	10	103	6.7	85	9
PR170	35	45	169	18	77	19	72	17
PO5	44	21	46	29	66	25	66	6.7
PY1	33	41	57	24	79	14	90	11

Sample preparation method development

Method development – SPE

Initial studies of the pigments' behaviour on solid phase extraction columns were performed by spiking a standard solution directly onto SPE columns. Three different polymeric columns were tested: Isolute ENV+, Oasis HLB and Phenomenex Strata-X. After application of the pigments, the columns were washed with water and eluted first with acetonitrile, then with ethyl acetate. Analysis of the SPE eluents showed that HLB and Strata-X gave the best recoveries.

Elution solvents were studied by spiking the pigments directly onto Strata-X columns. The columns were eluted with methanol, ethyl acetate, acetonitrile, dimethyl formamide, dichloromethane and acetonitrile:ethyl acetate 1:1. An aliquot of 1 mL of each sample was evaporated to near dryness and made up in 100 µL of acetonitrile before analysis. The samples were also analysed without concentration. As DMF has a very high boiling point, the DMF eluates were analysed without concentration.

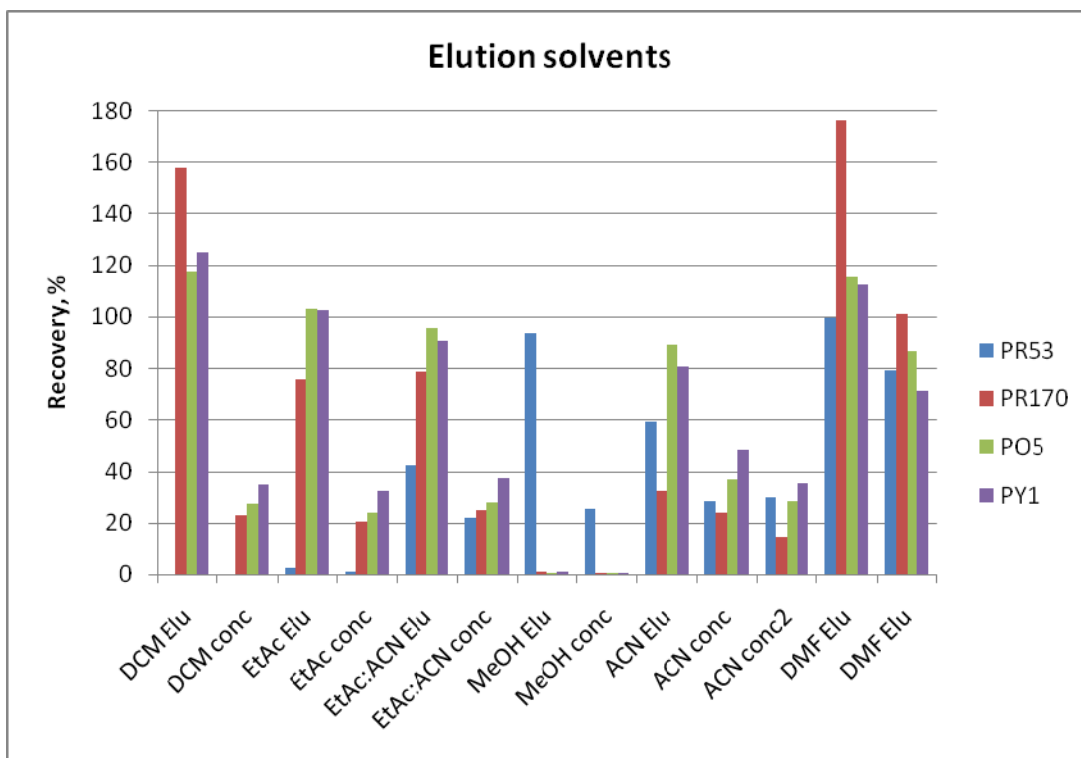


Figure 1: Elution efficiency of various solvents. Each solvent except DMF was analysed directly (Elu) and after ten times concentration (conc).

A 1:1 mixture of methanol and ethyl acetate was selected as elution solvent to yield good recoveries for all of the compounds.

Retention of pigments on the SPE columns was tested by spiking tap water and applying to Strata-X, ENV+ and HLB columns. The columns were eluted with 10 mL ethyl acetate:methanol 1:1. For PR53, the recovery was approximately 80 %, for PO5 and PY1 40 %. For PR170, the recovery was less than 10 %. The recoveries from HLB and Strata-X were similar, whereas the ENV+ gave recoveries less than 30 % for all four compounds. As the HLB columns have low particle size and tend to clog easily, the Strata-X columns were selected for the water samples.

To increase the recovery of PR170 the experiment was repeated, this time acidifying the samples to pH 3 with hydrochloric acid. Recovery of PR170 increased to 40% with acidification. For PR53, the recovery decreased to 60%. For PO5 and PY1, the recovery was unchanged.

In order to wash the Strata-X columns after application of the samples, a washing step using methanol:water in different ratios was tested. Methanol was selected as it was shown to be a poor eluent for three of the compounds. After spiking, the columns were eluted with solutions of 20 to 80 % methanol in water. At 50 % methanol, approximately 5 % of the PR53 was eluted, whereas at 45 %, none of the compounds were detected in the washing solution.

Method development – ASE

Initial method development for accelerated solvent extraction of pigments from the particle phase of water samples was performed by spiking standard solutions directly onto filters wetted with

purified water. The ASE unit was operated at 1500 psi and 100 °C. Heating and static times were 5 and 10 minutes, respectively, and the extraction cycle was repeated twice.

Based on results from the SPE method development, a set of solvents and solvent mixtures was selected for ASE extraction. Solvents tested include dichloromethane:methanol 1:1, ethyl acetate:acetonitrile 1:1 and pure acetonitrile. The two mixtures gave problems with phase separation of the extracts due to the water content of the samples. Addition of Na₂SO₄ to the cells to dry the extracts was unsuccessful, as the polar solvents dissolved the salt at the elevated temperature. Additionally, the Na₂SO₄ reduced the recoveries of the pigments. Adding Al₂O₃ to the cells for cleaner extracts was also attempted. As this gave a 90 % reduction in the recovery of PR53, this clean-up was omitted.